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(57) Abstract

A copolymer comprising an N–acylated derivative, and a composition comprising said copolymer and a polypeptide, said polypeptide comprising at least one effective ionogenic amine, wherein at least 50 percent, by weight, of said polypeptide present in said composition is ionically bound to said polymer.

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IONIC MOLECULAR CONJUGATES OF N-ACYLATED DERIVATIVES OF POLY(2-AMINO-2-DEOXY-D-GLUCOSE) AND POLYPEPTIDES

Cross Reference to Related Application

This application is a continuation-in-part of copending application, Application No. 08/929,363, filed September 9, 1997, which is a divisional application of Application No. 08/468,947, filed June 6, 1995.

Background of the Invention

Polymer drug delivery systems have been developed for the controlled release of pharmaceutical polypeptides. For example, synthetic polyesters such as poly(DL-lactic acid), poly(glycolic acid), poly(lactic-glycolic acid), and poly(ϵ-caprolactone) have been used in the form of microcapsules, films, or rods to release biologically active polypeptides. See e.g., U.S. Patent Nos. 4,767,628 and 4,675,189 and PCT Application No. WO 94/00148.

In addition to the synthetic polymeric chains, natural polymers and their derivatives have been used as components in similar sustained release compositions that dissociate by enzymatic degradation. One example of such natural polymers are those based on chitin, a poly(N-acetylglucosamine). However, since chitin is water insoluble, others have examined solubilizable derivatives which are based primarily on a partially deacetylated chitin, e.g., chitosan. See e.g., Sanford, P.A. et al., Eds., Advances in Chitin & Chitosan (1992). Although chitosan can be found in some fungi, the production of biodegradable chitosan is generally performed synthetically. See Mima, et. al., J. Appl. Polym. Sci. 28:1909-1917 (1983). Synthetic derivatives of chitosan have also been prepared to alter the polymer's *in vivo* biological characteristics. See Muzzarelli, et al., Carbohydrate Res. 207:199-214 (1980).

The use of chitin, as well as chitin derivatives, has been proposed in a number of drug delivery systems. See, e.g., European Patent Application Nos. 486,959, 482,649, 525,813 A1, and 544,000 A1; and U.S. Patent No. 5,271,945.

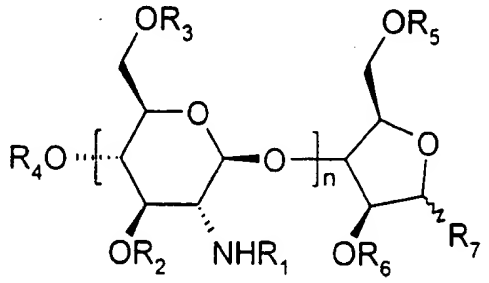
Summary of the Invention

In one aspect, the present invention features a copolymer including an N-acylated derivative of poly(2-amino-2-deoxy-D-glucose), wherein between 1 and 50 percent of the free amines of the poly(2-amino-2-deoxy-D-glucose) are acylated with a

first acyl group, the first acyl group is COE_1 where E_1 is selected from the group consisting of C_{3-33} carboxyalkyl, C_{3-33} carboxyalkenyl, C_{7-39} carboxyarylalkyl, and C_{9-39} carboxyarylalkenyl, and between 50 and 99 percent of the free amines of the poly(2-amino-2-deoxy-D-glucose) are acylated with a second acyl group, the second acyl group is COE_2 where E_2 is selected from the group consisting of C_{1-30} alkyl, C_{2-30} alkenyl, C_{6-37} arylalkyl, and C_{8-37} arylalkenyl, provided at least one of the free amines of the derivative is acylated with the first acyl group.

The copolymer preferably has a molecular weight of about 3,000 to 90,000 daltons. In other preferred embodiments, over 90 percent of the free amines of the poly(2-amino-2-deoxy-D-glucose) are acylated with either the first acyl group or the second acyl group. Preferably, between 10 and 30 percent of the free amine of the poly(2-amino-2-deoxy-D-glucose) are acylated with the first acyl group. Some of the free hydroxy groups (e.g., between 1 and 30 percent) of the derivative may be acylated with either the first acyl group or the second acyl group.

In a preferred embodiment, the copolymer is of the formula:



wherein:

R_1, for each individual repeat unit, is selected from the group consisting of first acyl group, second acyl group, and H;

R_2, for each individual repeat unit, is selected from the group consisting of first acyl group, second acyl group, and H;

R_3, for each individual repeat unit, is selected from the group consisting of first acyl group, second acyl group, and H;

R_4 is selected from the group consisting of first acyl group, second acyl group, and H;

R_5 is selected from the group consisting of first acyl group, second acyl group, and H;

R_6 is selected from the group consisting of first acyl group, second acyl group, and H;

R_7 is selected from the group consisting of COH and CH_2OR_8;

R_8 is selected from the group consisting of first acyl group, second acyl group, and H;

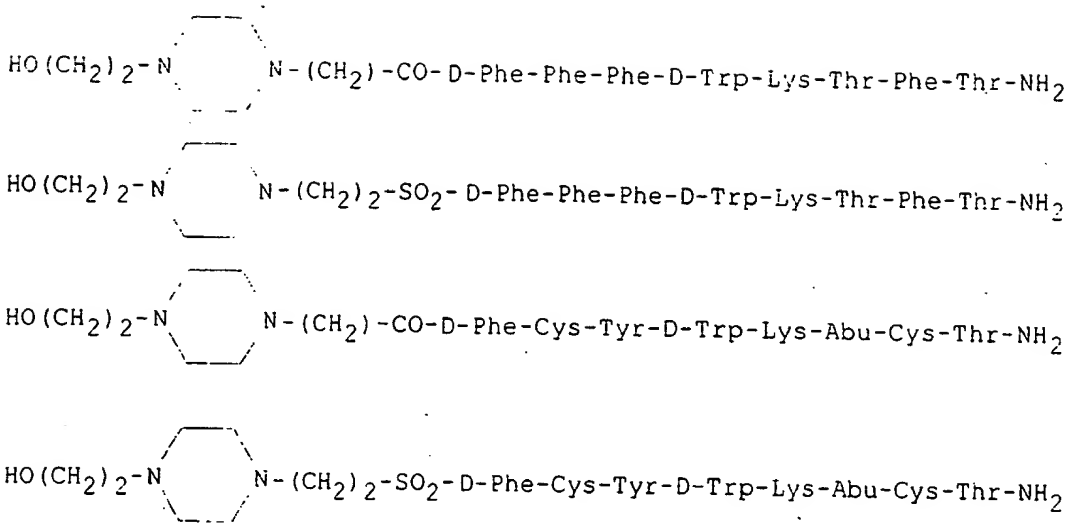
n is between 2 and 200; and

for between 1 and 50 percent of the repeat units, R_1 is first acyl group, and for between 50 and 99 percent of the repeat units, R_1 is second acyl group, provided that for at least one of the repeat units, R_1 is first acyl group.

The terms COE_1 and COE_2 stand for -C=O·E_1 and -C=O·E_2, respectively. The substituents carboxyalkyl, carboxyalkenyl, carboxyarylalkyl, and carboxyarylalkenyl may contain 1-4 carboxylic acid functionalities. Examples of the first acyl group include, but are not limited to, succinyl, 2-(C_{1-30} alkyl)succinyl, 2-(C_{2-30} alkenyl)succinyl, maleyl, phthalyl, glutaryl, and itaconyl. Examples of the second acyl group include but are not limited to, acetyl, benzoyl, propionyl, and phenylacetyl.

The present invention also features a composition including the above copolymer and a polypeptide, the polypeptide comprising at least one effective ionogenic amine, wherein at least 50 percent, by weight, of the polypeptide present in the composition is ionically bound to the polymer. Preferably, the composition comprises between 5 and 50 percent, by weight, of the polypeptide.

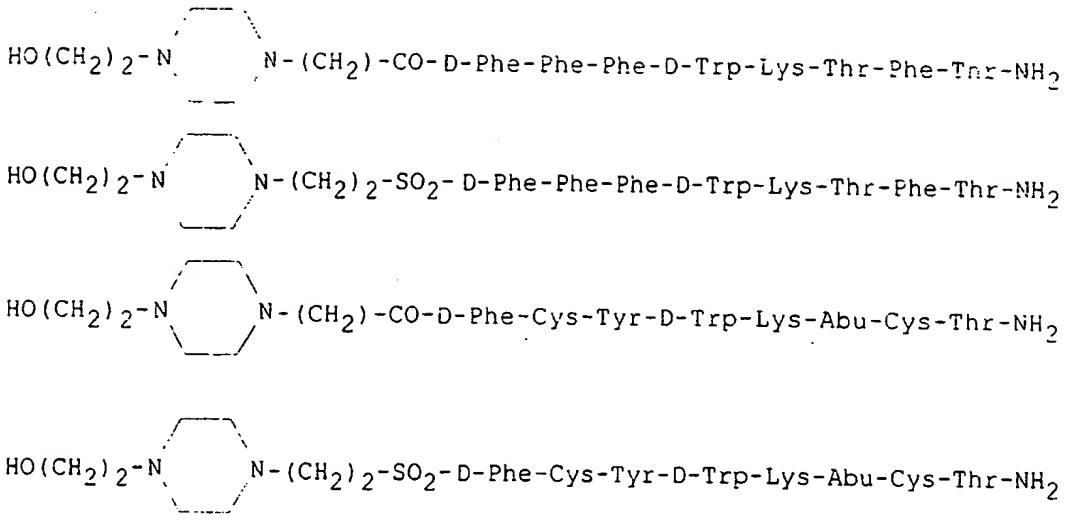
Preferred embodiments of the present invention include a copolymer wherein the first acyl group is succinyl and the second acyl group is acetyl and R_7 is COH or CH_2OH; a composition comprising said copolymer of claim 1 and H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 or a pharmaceutically acceptable salt thereof, wherein the two Cys are bonded by a disulfide bond, where at least 50 percent, by weight, of H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer; a composition comprising the foregoing copolymer and a peptide selected from the group consisting of



; and

or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof present in said composition is ionically bound to said copolymer; a composition comprising the foregoing copolymer and a peptide selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), and ([D-Nal^6]-LHRH, or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer; acomposition comprising the foregoing copolymer and parathyroid hormone, an analogue thereof or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of parathyroid hormone, an analogue thereof or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

Further preferred embodiments of the present invention include a copolymer wherein the first acyl group is glutaryl and the second acyl group is propionyl and R_7 is COH or CH_2OH; a composition comprising the foregoing copolymer and H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, wherein the two Cys are bonded by a disulfide bond, where at least 50 percent, by weight, of H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, present in said composition is ionically bound to said copolymer; a composition comprising the foregoing copolymer and a peptide selected from the group consisting of



; and

or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof present in said composition is ionically bound to said copolymer; a composition comprising the foregoing copolymer and a peptide selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-$NH_2{}^{10}$]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-$NH_2{}^{10}$]-LHRH(1-9)NHEt, ([des-Gly-$NH_2{}^{10}$]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6, des-Gly-$NH_2{}^{10}$]-LHRH(1-9)NHEt), ([D-Leu^6, des-Gly-$NH_2{}^{10}$]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-$NH_2{}^{10}$]-LHRH(1-9)NHEt), and ([D-Nal^6]-LHRH, or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer; and a composition comprising the foregoing copolymer and parathyroid hormone, an analogue thereof or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of parathyroid hormone, an analogue or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

Examples of suitable polypeptides include growth hormone releasing peptide (GHRP), luteinizing hormone-releasing hormone (LHRH), somatostatin, bombesin, gastrin releasing peptide (GRP), calcitonin, bradykinin, galanin, melanocyte stimulating hormone (MSH), growth hormone releasing factor (GRF), growth hormone (GH), amylin, tachykinins, secretin, parathyroid hormone (PTH), encephalon, endothelin, calcitonin gene releasing peptide (CGRP), neuromedins, parathyroid hormone related protein (PTHrP), glucagon, neurotensin, adrenocorticothrophic hormone (ACTH), peptide YY

(PYY), glucagon releasing peptide (GLP), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating peptide (PACAP), motilin, substance P, neuropeptide Y (NPY), TSH and biologically active analogs thereof. The term "biologically active analogs" is used herein to cover naturally occurring, recombinant, and synthetic peptides, polypeptides, and proteins having physiological or therapeutic activity. In general, the term covers all fragments and derivatives of a peptide, protein, or a polypeptide that exhibit a qualitatively similar agonist or antagonist effect to that of the unmodified, or naturally occurring peptide, protein, or polypeptide, e.g., those in which one or more of the amino acid residues occurring in the natural compounds are substituted or deleted, or in which the N- or C- terminal residues has been structurally modified. The term effective ionogenic amine refers to a free amine present on the polypeptide which is capable of forming an ionic bond with the free carboxylic groups on the copolymer.

Examples of other somatostatin analogs include, but are not limited to, the following somatostatin analogs which are disclosed in the above-cited references:

H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 acetate salt (also known as SOMATULINE™), where the two Cysteines are bonded by a disulfide bond;

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-β-Nal-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-β-Nal-NH_2;

H-D-β-Nal-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2;

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-OH;

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-OH;

H-Gly-Pen-Phe-D-Trp-Lys-Thr-Cys-Thr-OH;

H-Phe-Pen-Tyr-D-Trp-Lys-Thr-Cys-Thr-OH;

H-Phe-Pen-Phe-D-Trp-Lys-Thr-Pen-Thr-OH;

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol;

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-D-Trp-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Trp-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

Ac-D-Phe-Lys*-Tyr-D-Trp-Lys-Val-Asp*-Thr-NH_2 (an amide bridge formed between Lys* and Asp*);

Ac-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(Bu)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(Et)_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-L-hArg$(Et)_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;

Ac-L-hArg$(CH_2$-$CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NHEt;

Ac-hArg(CH_3, hexyl)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-hArg(hexyl$_2$)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;

Ac-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Propionyl-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys(iPr)-Thr-Cys-Thr-NH_2;

Ac-D-β-Nal-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Gly-hArg$(Et)_2$-NH_2;

Ac-D-Lys(iPr)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Ac-D-hArg$(Et)_2$-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-Cys-Lys-Asn-4-Cl-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-Phe-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-β-Nal-NH_2;

H-pentafluoro-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

Ac-D-β-Nal-Cys-pentafluoro-Phe-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

Ac-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-Phe-Cys-β-Nal-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2;

cyclo(Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Thr-N-Me-Phe);

cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Thr-Phe);

cyclo(Pro-Tyr-D-Trp-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe);

cyclo(Pro-Phe-L-Trp-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp(F)-Lys-Thr-Phe);

cyclo(Pro-Phe-Trp(F)-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Ser-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Thr-p-Cl-Phe);

cyclo(D-Ala-N-Me-D-Phe-D-Thr-D-Lys-Trp-D-Phe);

cyclo(D-Ala-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Phe);

cyclo(D-Ala-N-Me-D-Phe-D-Thr-Lys-D-Trp-D-Phe);

cyclo(D-Abu-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Tyr);

cyclo(Pro-Tyr-D-Trp-t-4-AchxAla-Thr-Phe);

cyclo(Pro-Phe-D-Trp-t-4-AchxAla-Thr-Phe);

cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe);

cyclo(N-Me-Ala-Tyr-D-Trp-t-4-AchxAla-Thr-Phe);

cyclo(Pro-Tyr-D-Trp-4-Amphe-Thr-Phe);

cyclo(Pro-Phe-D-Trp-4-Amphe-Thr-Phe);

cyclo(N-Me-Ala-Tyr-D-Trp-4-Amphe-Thr-Phe);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba-Gaba);

cyclo(Asn-Phe-D-Trp-Lys-Thr-Phe);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-NH$(CH_2)_4$CO);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-β-Ala);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-D-Glu)-OH;

cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe);

cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);

cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);

cyclo(Asn-Phe-Phe-D-Trp(F)-Lys-Thr-Phe-Gaba);

cyclo(Asn-Phe-Phe-D-Trp(NO_2)-Lys-Thr-Phe-Gaba);

cyclo(Asn-Phe-Phe-Trp(Br)-Lys-Thr-Phe-Gaba);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe(I)-Gaba);

cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Tyr(But)-Gaba);

cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;

cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;

cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Tpo-Cys)-OH;

cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-MeLeu-Cys)-OH;

cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Phe-Gaba);

cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-D-Phe-Gaba);

cyclo(Phe-Phe-D-Trp(5F)-Lys-Thr-Phe-Phe-Gaba);

cyclo(Asn-Phe-Phe-D-Trp-Lys(Ac)-Thr-Phe-NH-$(CH_2)_3$-CO);

cyclo(Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);

cyclo(Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);

cyclo(Orn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);

H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH_2;

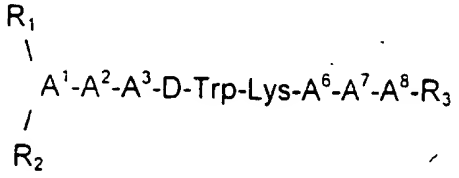
H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH_2;

H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH_2; and

H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH_2.

A disulfide bridge is formed between the two free thiols (e.g., Cys, Pen, or Bmp residues) when they are present in a peptide; however, the disulfide bond is not shown.

Also included are somatostatin agonists of the following formula:



wherein

A^1 is a D- or L- isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, β-Nal, β-Pal, Trp, Phe, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^2 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^3 is pyridyl-Ala, Trp, Phe, β-Nal, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^6 is Val, Ala, Leu, Ile, Nle, Thr, Abu, or Ser;

A^7 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^8 is a D- or L-isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

each R_1 and R_2, independently, is H, lower acyl or lower alkyl; and R_3 is OH or NH_2; provided that at least one of A^1 and A^8 and one of A^2 and A^7 must be an aromatic amino acid; and further provided that A^1, A^2, A^7 and A^8 cannot all be aromatic amino acids.

Examples of linear agonists to be used in a process of this invention include:

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH_2;

H-D-Phe-p-NO_2-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

H-D-Nal-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2;

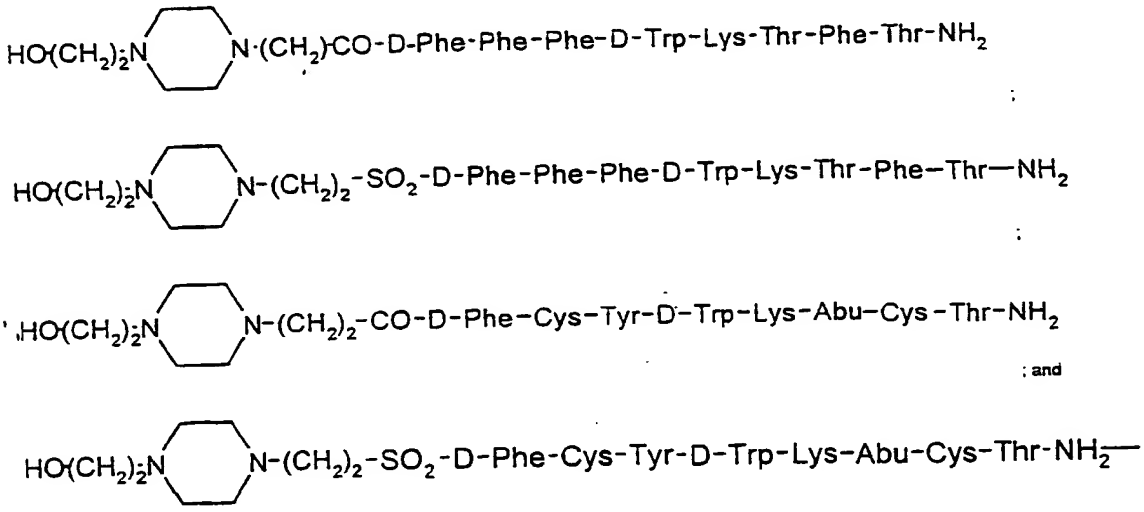
H-D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2; and

H-D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-β-D-Nal-NH_2.

If desired, one or more chemical moieties, e.g., a sugar derivative, mono or poly-hydroxy C_{2-12} alkyl, mono or poly-hydroxy C_{2-12} acyl groups, or a piperazine derivative, can be attached to the somatostatin agonist, e.g., to the N-terminus amino acid. See

PCT Application 88/02756, European Application 0 329 295, and PCT Application No. WO 95/04752. An example of somatostatin agonists which contain N-terminal chemical substitutions are:



or a pharmaceutically acceptable salt thereof.

Examples of specific LHRH analogues that can be incorporated in a conjugate or composition of this invention are TRYPTORELIN™ (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), buserelin ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), deslorelin ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, fertirelin ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), gosrelin ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), histrelin ([D-His(Bzl)^6,des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), leuprorelin (D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), lutrelin ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), nafarelin ([D-Nal^6]-LHRH) and pharmaceutically acceptable salts thereof.

The release of the polypeptide from the composition may be modified by changing the chemical structure of the composition. Increasing the molecular weight of the polymer will decrease the rate of peptide released from the conjugate. Increasing the number of carboxylic acid groups on the polymer will increase the amount of polypeptide ionically bound to the composition, and consequently, increase the amount of release of the peptide from the conjugate.

The release of the polypeptide may be further modulated through (a) treating the composition with soluble salts of divalent or polyvalent metallic ions of weak acids (e.g., calcium, iron, magnesium, or zinc); (b) coating the particles with a thin, absorbable layer made of a glycolide copolymer or silicone oil in a spherical, cylindrical or planar

configuration; or (c) microencapsulating the composition in an absorbable glycolide copolymer. In one embodiment, the composition comprises between 0.01 and 20 percent, by weight, of a polyvalent metal.

Depending on the choice of polypeptide, the compositions can be used to treat any number of disorders. For example, somatostatin, bombesin, GRP, LHRH, and analogs thereof, have been shown to treat various forms of cancer. Growth factors such as GH, GRF, and GHRP, and analogs thereof, have been shown to stimulate growth in both adolescents and the elderly. Calcitonin, amylin, PTH, and PTHrP, and analogs thereof, have been shown to treat osteoporosis and other bone disorders.

The compositions are designed for parenteral administration, e.g., intramuscular, subcutaneous, intradural, or intraperitoneal injection. Preferably, the compositions are administered intramuscularly.

The compositions of the invention can be in the form of powder or a microparticle to be administered as a suspension with a pharmaceutically acceptable vehicle (e.g., water with or without a carrier substance such as mannitol or polysorbate). The compositions may also be compounded in the form of a rod for parenteral implantation using a trocar, e.g., intramuscular implantation.

The dose of the composition of the present invention for treating the above-mentioned diseases or disorders varies depending upon the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such an amount of the composition as determined by the attending physician or veterinarian is referred to herein as a "therapeutically effective amount."

In another aspect, the present invention features a process of synthesizing a copolymer, the process comprising the steps of: reacting chitosan with a weak acid to produce a lower molecular weight polysaccharide; reacting between 1 and 50 percent of the free amines of the lower molecular weight polysaccharide with a first acylating agent, the first acylating agent selected from the group consisting of C_4-C_{34} polycarboxyalkane, C_4-C_{34} polycarboxyalkene, C_8-C_{40} polycarboxyarylalkane, C_{10}-C_{40} polycarboxyarylalkene, or an acylating derivative thereof; and reacting between 50 and 100 percent of the free amine of the lower molecular weight polysaccharide with a second acylating agent, the second acylating agent selected from the group consisting of C_{2-31} monocarboxyalkane, C_{3-31} monocarboxyalkene, C_{7-38} monocarboxyarylalkane, C_{9-35} monocarboxyarylalkene, or an acylating derivative thereof. The reaction of the

lower molecular weight polysaccharide with both the first acylating agent and the second acylating agent may be measured with an amine detecting agent (e.g., fluorescamine) to ensure that between 1 and 50 percent of the free amines of the lower molecular weight polysaccharide are acylated with the first acylating agent and between 50 and 99 percent of the free amines of the lower molecular weight polysaccharide are acylated with the second acylating agent. See, e.g., Bailey, P.D., An Introduction to Peptide Chemistry (Wiley, NY)(1990); Oppenheimer, H, et al. Archives Biochem. Biophys. 120:108-118 (1967); Stein, S, Arch. Biochem. Biophys. 155:203-212 (1973).

Reacting chitosan with the weak acid (e.g., nitrous acid) cleaves the polymer, thereby reducing its molecular weight (e.g., 2,500 - 80,000 daltons). In preferred embodiments, the first acylating group and the second acylating agent are reacted with the lower molecular weight polysaccharide successively, e.g., either the first acylating agent is reacted before the second acylating agent is reacted or the second acylating agent is reacted before the first acylating agent or simultaneously. As a result of the acylation of the free amines, some of the free hydroxy groups of the lower molecular weight polysaccharide may be acylated. The extent of the acylation of the free hydroxy groups may be altered by changing the pH or the solvents or agents used during the acylation reactions, or the acylating agents used.

Examples of acylating derivatives include, but are not limited to, anhydrides and N-acylated heterocycles (e.g., imidazoles and pyrazoles). See e.g., Bodansky, et al., The Practice of Peptide Synthesis, 87-150 (Springer-Verlag, 1984). The agents polycarboxyalkane, polycarboxyalkene, polycarboxyarylalkane, and polycarboxyarylalkene or acylating derivatives thereof contain, or originate from reactants containing, 2-5 carboxylic acid functionalities. The substituents monocarboxyalkane, monocarboxyalkene, monocarboxyarylalkane, and monocarboxyarylalkene contain, or originate from reactants containing, only a single carboxylic acid group. Examples of first acylating agents include, but are not limited to, succinic anhydride, 2-(C_{1-30} alkyl)succinic anhydride, 2-(C_{2-30} alkenyl)succinic anhydride, maleic anhydride, glutaric anhydride, itaconic anhydride, and phthalic anhydride. Examples of second acylating agents include, but are not limited to, acetic anhydride, benzoic anhydride, N,N'-diacetyl-3,5-dimethylpyrazole, N,N'-diacetylimidazole, phenylacetic anhydride, propionic anhydride, and butyric anhydride.

In yet another aspect, the present invention features a process of synthesizing a composition, the process comprising the steps of: reacting chitosan with a weak acid to produce a lower molecular weight polysaccharide; reacting between 1 and 50 percent of the free amines of the lower molecular weight polysaccharide with a first acylating agent, the first acylating agent selected from the group consisting of C_4-C_{34} polycarboxyalkane, C_4-C_{34} polycarboxyalkene, C_8-C_{40} polycarboxyarylalkane, C_{10}-C_{40} polycarboxyarylalkene, or an acylating derivative thereof; reacting between 50 and 100 percent of the free amine of the lower molecular weight polysaccharide with a second acylating agent, the second acylating agent selected from the group consisting of C_{2-31} monocarboxyalkane, C_{3-31} monocarboxyalkene, C_{7-38} monocarboxyarylalkane, C_{9-35} monocarboxyarylalkene, or an acylating derivative thereof; neutralizing the acylated lower molecular weight polysaccharide with a base; and mixing the neutralized lower acylated molecular weight polysaccharide with a polypeptide salt, wherein the polypeptide salt comprises at least one ionogenic amine, to form a polypeptide-copolymer ionic conjugate.

The neutralization step preferably renders the lower molecular weight polysaccharide emulsifiable or soluble in water. In preferred embodiments, the base is an inorganic base (e.g., sodium hydroxide). The polypeptide salt is preferably a weak acid salt (e.g., acetate, lactate, or citrate). The ionic conjugate can be isolated by filtering or by centrifuging the resulting mixture.

The conjugates of the invention can easily be made into injectable microspheres or microparticles, and implantable films or rods, without the need to utilize processing that entails multiphase emulsions. Preferably, the microparticles are manufactured by (a) dissolving the composition in an aprotic, water miscible organic solvent; (b) mixing the organic solvent in water; and (c) isolating the microparticles from the water. In preferred embodiments, the organic solvent is chosen from the group of acetone, acetonitrile, tetrahydrofuran, dimethylformamide, and dimethyl ethylene glycol.

Other features and advantages of the present invention will be apparent from the detailed description and from the claims.

Detailed Description of the Invention

The synthesis and use of the copolymer and copolymer-polypeptide ionic conjugates of this invention are well within the ability of a person of ordinary skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Example 1: Depolymerization of Chitosan

Chitosan (Protan, Inc., Portsmouth, NH) is dissolved in aqueous acetic acid by stirring with a mechanical stirrer for one day. Nitrogen gas is bubbled through the solution, while an aqueous solution of sodium nitrite is added. After a half hour, the solution is filtered through a sintered glass funnel, under reduced pressure, to remove insoluble particles which are present in the initial chitosan solution. To the filtered solution is added an aqueous solution of NaOH, and the solution is vigorously stirred in methanol to precipitate the polymer. The resulting precipitate is then filtered and alternately washed five times with water and methanol. The precipitate is then dried in a vacuum oven at 60°C for two days. The depolymerized chitosan comprises an aldehyde group at one end of the chain. The aldehyde end group may be reduced to a primary hydroxyl group by reaction $NaBH_4$. The depolymerized product can be analyzed by gel permeation chromatography (GPC) to determine both its molecular weight and molecular weight distribution (MWD) in comparison to Pullulan reference standards. NMR (nuclear magnetic resonance) and IR (infra-red) studies can be used to determine the amount of N-acetylation on the depolymerized product.

Example 2: Partial Succinylation of Depolymerized Chitosan

The depolymerized chitosan from Example 1 is dissolved in 0.1M aqueous acetic acid. To this solution, methanol is added followed by the addition of a solution of succinic anhydride in acetone. The resulting solution is stirred at room temperature for 24 hours. Upon completion of the succinylation, the solution is then precipitated into aqueous acetone. The resulting precipitate is collected by centrifugation and washed five times with methanol. The precipitate is then dissolved in 0.5M KOH and dialyzed against water to a pH of 7. The dialyzed solution is then concentrated under reduced pressure, precipitated in aqueous acetone, and dried in a vacuum oven at 60°C.

To obtain variable levels of succinylation, the extent of the reaction can be monitored as the acylation proceeds by analyzing for number of unacylated amine

groups. The number of unacylated amine groups can be determined by quenching a withdrawn sample of the reaction mixture with an amine detecting agent (e.g., flouorescamine). The amount of amine present can be measured spectrophoretically using a standard curve for the copolymer. Additionally, succinic anhydride, thus, can be added successively until the desired acylation percentage is achieved. The exact degree of succinylation of the purified product can be determined using ^{1}H NMR spectroscopy and conductometric titration.

Example 3: Acetylation of the N-succinylated chitosan

The partial succinylated sample from Example 2 is dissolved in 0.1M aqueous acetic acid. To this solution, methanol and acetic anhydride is then added, and the reaction mixture is stirred at room temperature for one day. This solution is then precipitated in aqueous acetone. The resulting precipitate is collected by centrifugation and washed five times with methanol. The precipitate is then dissolved in 0.1N KOH and is dialyzed against water to a pH of 7. The final solution is lyophilized to obtain the final product. The acylation procedure can be measured spectrophoretically as discussed in Example 2, and the exact degree of acylation of the purified product can be determined using ^{1}H NMR spectroscopy and conductometric titration.

Example 4: Preparation of poly(N-acyl-D-glucosamine)-peptide ionic conjugate

The N-succinylated chitosan potassium salt of Example 3 is dissolved in water. An aqueous solution of the acetate salt of the somatostatin polypeptide analog SOMATULINE™ (D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2; Kinerton, Dublin, Ireland) is added to the stirred polymer solution. A precipitate forms and is filtered and dried in a vacuum oven at 40°C.

The polypeptide content of the resulting ionic conjugate can be determined by the difference between the amount of initial peptide added and the amount of free residual peptide contained in the filtrate and rinse solution. The peptide content of the resulting ionic conjugate can be determined by comparing the carbon/nitrogen ratio of the initial N-succinylated chitosan with that of the resulting ionic conjugate. GPC analysis can be used to determine molecular weight and MWD, differential scanning calorimetry (DSC) to determine thermal properties and NMR and IR for chemical identity.

Example 5: Homogeneous Depolymerization of Chitosan

Chitosan (Aldrich, Sigma-Aldrich Co. Ltd., Gillingham, Dorset, England, high molecular weight, 10g) was dissolved in 1L of 0.1M aqueous acetic acid (acetic acid, min. 99.8%, Riedel-de Haën, article number 33209) in a 2L glass beaker with stirring at about 144 rpm using a Heidolph mechanical stirrer (model RZR 2102, Kelheim, Germany). Dissolution was complete within ~4 hours. The inherent viscosity, η_{inh}, of the final depolymerized chitosan was shown to be dependent on the concentration of sodium nitrite and the time given for depolymerization, t_{depoly}, (Table 1). Inherent viscosity, η_{inh}, was determined using a Cannon-Fenske routine Ubbelodhe viscometer (Poulten Selfe & Lee ltd., number 50 of constant 0.003890(mm^2/s)/s at 40°C) with 0.1M acetic acid as solution.

Table 1

$NaNO_2$ (g)	$T_{depolym}$ (min)	Yield (%)	η_{inh} (DL/g)
0.76	35	90.8	1.52
0.76	45	87.6	0.98
0.76	55	85.1	0.65
0.152	30	76.7	0.33
0.304	30	22.0	0.23
0.304	90	No ppt.	-

Sodium nitrite (Aldrich, Sigma-Aldrich Co. Ltd., Gillingham, Dorset, England) in 5-20ml de-ionized (DI) water (depending on the mass) was added to the solution. After the required depolymerization time, the solution was filtered as quickly as possible through a sintered glass funnel (25-50μ, Ace Glass Incorporated, Vineland, N.J.) to remove insoluble matter. To the filtered solution was added NaOH (Aldrich) in DI water (ranging from 4.5g in 100ml to 20g in 400ml) to quench the depolymerizing action of $NaNO_2$. Solution was then added to vigorously stirred methanol (Labscan, HPLC grade, 300ml) to precipitate the polymer. Suspension was spun at 4,000rpm at 4°C for 35min using a Sorvall RC 5B plus centrifuge. After spinning, the supernatant was decanted off and precipitate was washed with a water/methanol (Labscan, AR grade) mixture (1L, 80:20). Suspension was centrifuged as before, supernatant was again decanted off and depolymerized chitosan was lyophilised in an Edwards Super Modulyo lyophiliser for two days following overnight refrigeration. The depolymerized chitosan was further dried for 1 day in a vacuum oven (Bioblock Scientific, Strasbourg, -22mmHg at 30°C).

Example 6: Heterogeneous Depolymerization

Chitosan (18.0g, as before) and $NaNO_2$ (as before) were added to a 1L glass beaker. Trifluoroacetic acid solution (Riedel-de Haën, 23ml in 600ml DI water, 0.5M) was added to the beaker and the mixture was stirred using a Heidolph mechanical stirrer (as before). Considerable fizzing was observed on addition of the TFA solution. The solution was filtered on a sintered glass funnel (as before). NaOH solution (13.3g in 165ml DI water) was added to the filtered solution. The resulting solution was then added to vigorously stirred methanol (Labscan, HPLC grade, 300ml). Centrifugation and washing was carried out as per homogeneous depolymerization. Table 2 gives results from a series of depolymerization experiments.

Table 2

$NaNO_2$ (g)	$T_{depolym}$ (min)	Yield (%)	η_{inh} (DL/g)
9.0	15	24.0	0.19
9.0	23	20.9	0.13
9.0	45	16.3	0.11
4.5	15	69.5	0.30

With the heterogeneous method, dissolution and depolymerization take place simultaneously making it a faster method. Both methods gave similar yields (Table 1; 0.33DL/g with a yield of 76.7% and 0.30DL/g with a yield of 69.1%) but with the heterogeneous method larger quantities of chitosan can be used; 18g as opposed to 10g. The dried depolymerized chitosan samples (from examples 5 and 6) with inherent viscosity values in the range 0.23-1.51DL/g were analysed by ^{13}C NMR in aqueous CD_3COOD using a Bruker Spectrospin 400 NMR spectrometer. Chemical shifts of carbons C_1 to C_6 are given in Table 3. The chemical shift of a particular carbon increases with the inherent viscosity.

Table 3

C Type	Shift (ppm)
C_1	96.12-99.30
C_2	54.58-57.44
C_3	75.20-78.69
C_4	73.55-76.41
C_5	68.90-71.80
C_6	58.74-61.77

Elemental analysis was carried out on the depolymerized chitosan samples from examples 5 and 6 (Table 4).

Tabl 4

	η_{inh} (DL/g)	% Nitrogen
Series of Depolymerized Chitosan samples	0.11	3.07
	0.13	3.50
	0.19	3.40
	0.30	5.42
	0.33	5.87
	0.98	6.78
Low mol. Wt. Chitosan	8.76	7.11
High mol. Wt. Chitosan	48.50	7.42

The amino content, that is the fraction of chitosan repeating units containing amino groups was obtained by a metachromatic titration using acid red 88 (Aldrich, dye content ~75%) by following the method outlined by Gummow and Roberts (Beryl. D. Gummow, George A.F. Roberts, Makromol. Chem. 186, 1239-1244 (1985), the contents of which are incorporated herein). Amino content values are given in Table 5.

Table 5

η_{inh} (DL/g)	Amino Content
48.9 (Aldrich High mol. wt. Chitosan)	0.83
8.76 (Aldrich Low mol. wt. Chitosan)	0.78
0.98	0.74
0.30	0.56

From % nitrogen values and the amino content values of a series of depolymerized chitosan samples in Tables 4 and 5, it is evident that a decrease in η_{inh} is accompanied by a decrease in the amino content indicating deamination with depolymerization.

Calculations For Glutarylation/Propionylations:

The masses of glutaric and propionic anhydrides required for a glutarylation/ propionylation reaction are dependent on the desired molar ratio between the two anhydrides, the mass and amino content of depolymerized chitosan used. General equations for the masses of anhydrides for stoichiometric glutarylation/propionylation are given here:

Mass of Glutaric Anhydride (GA) required= Desired GA Fraction x Mass Chitosan x Amino Content x 114.1* /161**

*F.W. (GA)

**161 = F.W. repeating unit of Chitosan

Mass of Propionic Anhydride (PA) required= Desired PA Fraction x Mass Chitosan x Amino Content x 130.14***/161

***F.W. (PA)

Example 7A: Glutarylation/Propionylation of Depolymerized Chitosan

Depolymerized chitosan from example 5 with an inherent viscosity of 1.51DL/g was dissolved in 0.1M acetic acid (4.01g in 150ml). The amino content of this sample was not known at the time but it can be assumed that it is between 0.74 for depolymerized chitosan of inherent viscosity 0.98DL/g and 0.78 for low mol. wt. chitosan from Aldrich (Table 5). Glutaric anhydride (Aldrich, 95%, 6.0g) and propionic anhydride (Aldrich, 99+%m, 6.0g) with glutaric anhydride at an approximately 5.7 fold excess and propionic anhydride at an approximately 5.0 fold excess in acetone (Labscan, Dublin, Ireland, AR grade, 29.9ml, 23.62g) solution were added to the chitosan solution and left stirring overnight. Resulting solution which was gel-like in nature was poured into acetone (Labscan, AR grade, 200ml) to induce precipitation. Dispersion was spun at 4000rpm at about 4°C for about 25min. After spinning, supernatant was washed with methanol (Labscan, HPLC grade, 600ml) and spun as before. Supernatant was decanted off and product was lyophilized following overnight refrigeration. Because of the high excess of anhydride used, the lyophilized product was washed by redissolving in 0.2M NaOH solution, filtering to remove insoluble matter and precipitation in methanol (Labscan, HPLC grade, 300ml). After spinning at 4000rpm at about 4°C for about 25min, supernatant was decanted off and the product was dried by lyophilization (2 days) and vacuum dried for 1 day. % Nitrogen in the final product as determined by elemental analysis was 3.92%.

Example 7B: Glutarylation/Propionylation of Depolymerized Chitosan

Depolymerized chitosan from example 5 with an inherent viscosity of 0.98DL/g was dissolved in 0.1M acetic acid (1.23g in 46ml). The amino content of this sample was calculated to be 0.74 (Table 5). Glutaric anhydride (Aldrich, 95%, 1.33g) and propionic anhydride (Aldrich, 99+%m 1.33g) with glutaric anhydride at an approximately 3.8 fold excess and propionic anhydride at an approximately 3.3 fold excess in acetone (Labscan, AR grade, 10.1ml, 8g) solution was added to the chitosan solution and left

stirring overnight. Resulting solution was poured into acetone (Labscan, AR grade, 80ml) to induce precipitation. Dispersion was spun at 4000rpm at about 4°C for about 25min. After spinning, supernatant was washed with methanol (Labscan, HPLC grade, 600ml) and spun as before. Supernatant was decanted off and product was lyophilized following overnight refrigeration and then vacuum oven dried (1 day). % Nitrogen of this product as determined from elemental analysis was 5.11%.

Example 7C: Glutarylation/Propionylation of Depolymerized Chitosan

Depolymerized chitosan from example 5 with an inherent viscosity of 0.98DL/g was dissolved in 0.1M acetic acid (4.02g in 150ml). The amino content of this sample was calculated to be 0.74 (Table 5). Glutaric anhydride (Aldrich, 95%, 4.01g) and propionic anhydride (Aldrich, 99+%m 4.05g) with glutaric anhydride at an approximately 3.8 fold excess and propionic anhydride at an approximately 3.3 fold excess in acetone (Labscan, AR grade, 29.4ml, 23.2g) solution was added to the chitosan solution and left stirring overnight. Resulting solution was poured into acetone (Labscan, AR grade, 200ml) to induce precipitation. Dispersion was spun at 4000rpm at about 4°C for about 25min. After spinning, supernatant was washed with methanol (Labscan, HPLC grade, 600ml) and spun as before. Supernatant was decanted off and product was lyophilized following overnight refrigeration. Because of the high excess of anhydride used, the lyophilized product was washed by redissolving in 0.2M NaOH solution, filtering to remove insoluble matter and precipitation in methanol (Labscan, HPLC grade, 300ml). After spinning at 4000rpm at about 4°C for about 25min, supernatant was decanted off and the product was dried by lyophilization (2 days) and vacuum dried for 1 day. % Nitrogen of this product as determined from elemental analysis was 5.11%.

Example 7D: Glutarylation/Propionylation of Depolymerized Chitosan

Depolymerized chitosan from example 6 with an inherent viscosity of 0.30DL/g was dissolved in 0.1M acetic acid (4.01g in 150ml). The amino content of this sample was calculated to be 0.56. Glutaric anhydride (Aldrich, 95%, 3.0g) and propionic anhydride (Aldrich, 99+%, 1.0g) in acetone (Labscan, Dublin, Ireland, AR grade, 29.9ml, 23.6g) solution was added to the chitosan solution and left stirring overnight. Resulting solution was poured into acetone (Labscan, AR grade, 200ml) to induce precipitation. Dispersion was spun at 4000rpm at about 4°C for about 25min. After spinning, supernatant was washed with methanol (Labscan, HPLC grade, 600ml) and spun as before. Supernatant was decanted off and product was lyophilized for 2 days

following overnight refrigeration and vacuum dried for 1 day. % Nitrogen of this product as determined from elemental analysis was 5.29%.

Glutarylation/Propionylation – A Kinetic Study

6.51 grams of chitosan from example 5 of inherent viscosity, 0.98DL/g and amino content of 0.74 were dissolved in 0.1M aqueous acetic acid (225ml). A molar ratio of propionic anhydride to glutaric anhydride of 4 was desired in the final product. Glutaric anhydride (Aldrich, 95%, 1.443g) was dissolved in methanol (10ml) (Labscan, Dublin, Ireland, HPLC grade) and the solution was added to the chitosan solution with stirring at room temperature. After about 2 hours, a 40ml aliquot of the reaction mixture was precipitated in acetone (Labscan, Dublin, Ireland, A.R. grade) spun at 2900rpm at about 4°C for about 25min. Precipitate was washed with methanol (Labscan, HPLC grade) and dried. Another 40ml aliquot was taken after 4 hours, precipitated, washed and dried as before. Immediately after the 40ml aliquot was taken (4 hours), propionic anhydride solution (Aldrich, 99+%, 2.6489g in methanol (Labscan, HPLC grade, 10ml)) was added to the reaction mixture. After a further 2 hours reaction (equivalent to a total reaction time of 6 hours from the addition of the glutaric anhydride solution), the entire mixture was precipitated in acetone (Labscan, AR grade, 330ml), dispersion was spun at 4000rpm at about 4°C for about 30min in a Sorvall RC plus centrifuge. After spinning, supernatant was decanted off and cake was washed twice with methanol (Labscan, HPLC grade, 400ml), lyophilized and vacuum oven dried. A metachromatic titration as mentioned in examples 5 and 6 was carried out on the three modified chitosan samples taken at 2 hours, 4 hours and finally 6 hours. The amino content of these three samples is given in Table 6.

Table 6

Time (hours)	Amino Content
2	0.91
4	0.43
6	0.10

Example 8A: Preparation of Poly(N-propionylated, N-glutarylated, N-acetylated-D-glucosamine)-Peptide Ionic Conjugate

1.0038 grams of product from example 7A was dissolved in 20ml 0.2M NaOH solution. 450µl of acetic acid was added to the glutarylated/propionylated solution to

bring the pH down to ~6. Modified chitosan solution was then added slowly to a solution of 122.9mg of H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 acetate salt (also known as SOMATULINE™), where the two Cysteines are bonded by a disulfide bond (Kinerton ltd., Blanchardstown, Dublin, Ireland, B/N 93K2505DL3, Acetate =12.37%, potency = 82.68%) in 3.73g DI water and precipitation was observed. The precipitate is also known as a conjugate of the polymer and drug. Dispersion was spun at 2900rpm at about 4°C for about 25min using a Sorvall RT 6000 centrifuge. Supernatant was decanted off and retained for further manipulation. Precipitate was placed in a refrigerator. Acetic acid was added to the retained supernatant until the pH fell to ~4. Further precipitation ensued. Dispersion was spun as before and then stored in fridge. Precipitate from first and second centrifuges were lyophilized, vacuum oven dried and yields obtained were respectively 31.7% (356.9mg) and 36.4% (409.6mg) with a combined yield of 68.1%. Elemental analysis was carried out on the combined product. % Nitrogen as determined from elemental analysis for the combined conjugate was 4.60% and using the % nitrogen of 3.92% for product from example 7A, the % loading of SOMATULINE™ was calculated to be 6.7%.

In vivo assay: Conjugate of example 8A was suspended in saline containing Tween® 20 (1%) and injected at 7.5mg peptide equivalent per rat. SOMATULINE™ levels in rat plasma induced by this conjugate were between a maximum value of 636,757+/-124,759 pg/ml and a minimum value of 863+/-145pg/ml over a 15 day period, see Table 7.

Example 8B: Preparation of Poly(N-propionylated, N-glutarylated, N-acetylated-D-glucosamine)-Peptide Ionic Conjugate

1.0010 grams of product from example 7A was dissolved in 12ml 0.2M NaOH solution. 250µl of acetic acid was added to the glutarylated/propionylated solution to bring the pH down to ~6. Modified chitosan solution was then added slowly to a solution of 120.3mg of SOMATULINE™ (Kinerton ltd., Blanchardstown, Dublin, Ireland, B/N 93K2505DL3, Acetate =12.37%, potency = 82.68%) in 2.87g DI water and precipitation was observed. Because of the lower volume of NaOH solution used, the resulting solution was extremely viscous. Dispersion was spun at 2900rpm at about 4°C for about 25min using a Sorvall RT 6000 centrifuge. Supernatant was decanted off and retained for further manipulation. Precipitate was placed in a refrigerator. Acetic acid was added to the retained supernatant until the pH fell to ~4. Further precipitation ensued.

Dispersion was spun as before and then stored in a refrigerator. Precipitate from first and second centrifuges were lyophilized, vacuum oven dried and yields obtained were respectively 53.5% (600.4mg) and 20.9% (234.8mg) with a combined yield of 74.4%. Elemental analysis was carried out on the combined product. % Nitrogen as determined from elemental analysis for the combined conjugate was 4.30% and using the % nitrogen of 3.92% for product from example 7A, the % loading of SOMATULINE™ was calculated to be 4.0%.

In vivo assay: Conjugate was suspended in saline containing Tween® 20 (1%) and injected at 7.5mg peptide equivalent per rat. SOMATULINE™ levels in rat plasma induced by this conjugate were between a maximum value of 545,367+/-69,445 pg/ml and a minimum value of 1134+/-325pg/ml over a 15 day period, see Table 7.

Example 8C: Preparation of Poly(N-propionylated, N-glutarylated, N-acetylated-D-glucosamine)-Peptide Ionic Conjugate

1.0190 grams of example 7B was dissolved in 20ml 0.2M NaOH solution. 200µl of acetic acid was added to the glutarylated/propionylated solution to bring the pH down to ~6. Modified chitosan solution was then added slowly to a solution of 102.1mg of SOMATULINE™ (Kinerton ltd., Blanchardstown, Dublin, Ireland, B/N 93K2505DL3, Acetate =12.37%, potency = 82.68%) in 2.56g DI water and precipitation was observed. Dispersion was spun at 2900rpm at about 4°C for about 25min using a Sorvall RT 6000 centrifuge. Supernatant was decanted off and retained for further manipulation. Precipitate was placed in refrigerator. Acetic acid was added to the retained supernatant until the pH fell to ~4. Further precipitation ensued. Dispersion was spun as before and then stored in a refrigerator. Precipitate from first and second centrifuges were lyophilized, vacuum oven dried and the combined yield obtained was 74% (827.1mg). % Loading of SOMATULINE™ in this conjugate was taken to be similar to the % Loading of SOMATULINE™ example 4D i.e., 14%.

In vivo assay: Conjugate was suspended in saline containing Tween® 20 (1%) and injected at 7.5mg peptide equivalent per rat. SOMATULINE™ levels in rat plasma induced by this conjugate were between a maximum value of 168,141+/- 90,972 pg/ml and a minimum value of 1000pg/ml over a 9 day period, see Table 7.

Example 8D: Preparation of Poly(N-propionylated, N-glutarylated, N-acetylated-D-glucosamine)-Peptide Ionic Conjugate

1.0149 grams of example 7C was dissolved in 15ml 0.05M NaOH solution. The molarity of the NaOH in this example is lower than that of example 8C. 200µl of acetic acid was added to the glutarylated/propionylated solution to bring the pH down to ~6. Modified chitosan solution was then added slowly to a solution of 125.2mg of SOMATULINE™ (Kinerton ltd., Blanchardstown, Dublin, Ireland, B/N 93K2505DL3, Acetate =9.37%, potency = 80.68%) in 3.0g DI water and precipitation was observed. Dispersion was spun at 2900rpm at about 4°C for about 25min using a Sorvall RT 6000 centrifuge. Supernatant was decanted off and retained for further manipulation. Precipitate was placed in fridge. Acetic acid was added to the retained supernatant until the pH fell to ~4. Further precipitation ensued. Dispersion was spun as before and then stored in fridge. Precipitate from first and second centrifuges were lyophilized, vacuum oven dried and the combined yield obtained was only 24% (270mg). Elemental analysis was carried out on the combined product. % Nitrogen as determined from elemental analysis for the combined conjugate was 6.34% and using the % nitrogen of 5.11% for product from example 7C, the % loading of SOMATULINE™ was calculated to be 14.0%.

In vivo assay: Conjugate was suspended in saline containing Tween® 20 (1%) and injected at 7.5mg peptide equivalent per rat. SOMATULINE™ levels in rat plasma induced by this conjugate were between a maximum value of 192,419+/-112,621 pg/ml and a minimum value of 1000/ml over a 12 day period, see Table 7.

Example 8E: Preparation of Poly(N-propionylated, N-glutarylated, N-acetylated-D-glucosamine)-Peptide Ionic Conjugate

2.0 grams of example 7D was dissolved in 28ml 0.05M NaOH solution. Chitosan solution was then added slowly to a solution of 246.0mg of SOMATULINE™ (Kinerton ltd., Blanchardstown, Dublin, Ireland, B/N 93K2505DL3, Acetate =9.37%, potency = 80.68%) in 6.0g DI water and precipitation was observed. Dispersion was spun at 2900rpm at about 4°C for about 25min using a Sorvall RT 6000 centrifuge. Supernatant was decanted off. Precipitate was washed with 24ml DI water and spun as before. Precipitate was then placed in a refrigerator. Elemental analysis was carried out on the product. % Nitrogen as determined from elemental analysis for the conjugate was 6.6%

and using the % nitrogen of 5.29% for product from example 7D, the % loading of SOMATULINE™ was calculated to be 15%.

In vivo assay: Conjugate was suspended in saline containing Tween® 20 (1%) and injected at 3.75mg peptide equivalent per rat. SOMATULINE™ levels in rat plasma induced by this conjugate were between a maximum value of 145,429±122,743pg/ml and a minimum value of 500±159/ml over a 10 day period, see Table 7.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the claims.

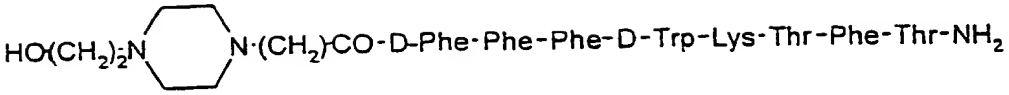
CLAIMS

What is claimed is:

1. A copolymer comprising an N-acylated derivative of poly(2-amino-2-deoxy-D-glucose), wherein between 1 and 50 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) are acylated with glutaryl and between 50 and 99 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) are acylated with propionyl and a terminal monomer of said N-acylated derivative of poly(2-amino-2-deoxy-D-glucose) contains COH or CH_2OH.

2. A composition comprising a copolymer and a peptide, wherein said copolymer comprises an N-acylated derivative of poly(2-amino-2-deoxy-D-glucose) having between 1 and 50 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) acylated with succinyl, between 50 and 99 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) acylated with acetyl provided that at least one of the free amines of said poly(2-amino-2-deoxy-D-glucose) is acylated with succinyl, and a terminal monomer of said N-acylated derivative of poly(2-amino-2-deoxy-D-glucose) containing COH or CH_2OH, and wherein said peptide comprises H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 or a pharmaceutically acceptable salt thereof, having the two Cys of said peptide bonded by a disulfide bond and at least 50 percent by weight, of the H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 peptide, or a pharmaceutically acceptable salt thereof, present in said composition ionically bound to said copolymer.

3. A composition comprising said copolymer of claim 2 and a peptide wherein said peptide is selected from the group consisting of



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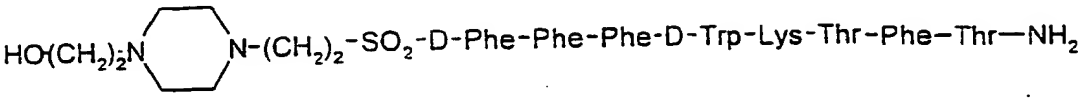

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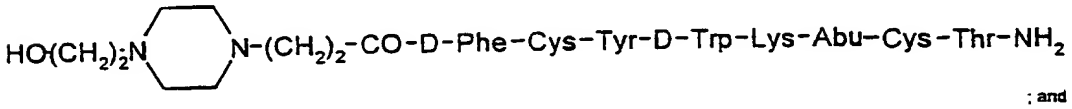
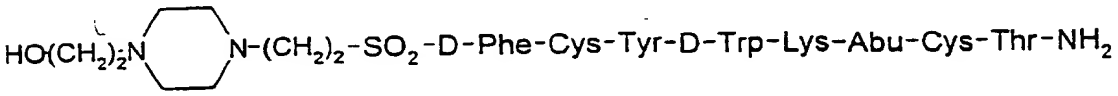


; and

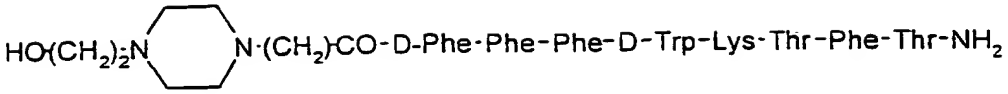


or a pharmaceutically acceptable salt thereof, having at least 50 percent, by weight, of said peptide, or a pharmaceutically acceptable salt thereof, present in said composition, ionically bound to said copolymer.

4. A composition comprising said copolymer of claim 2 and a peptide wherein said peptide is selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, ([des-Gly-NH_2^{10}-]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6,des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), (D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Nal^6]-LHRH) or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by weight, of said peptide, or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

5. A composition comprising said copolymer of claim 1 and H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, wherein the two Cys are bonded by a disulfide bond and at least 50 percent, by weight, of H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, present in said composition is ionically bound to said copolymer.

6. A composition comprising said copolymer of claim 1 and a peptide selected from the group consisting of



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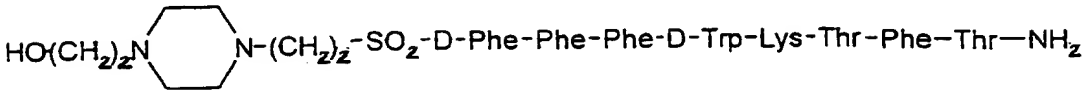

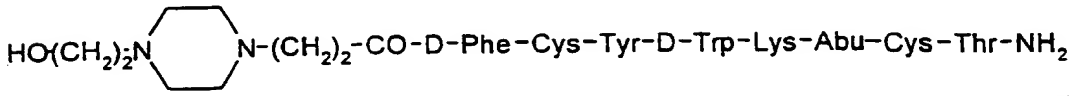

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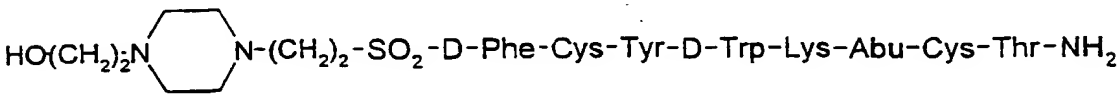

or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof present in said composition is ionically bound to said copolymer.

7. A composition comprising said copolymer of claim 1 and a peptide selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6,des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), (D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Nal^6]-LHRH) or a pharmaceutically acceptable salts thereof, wherein at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

8. A composition comprising said copolymer of claim 2 and parathyroid hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by weight, of said parathyroid hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

9. A composition comprising said copolymer of claim 1 and parathyroid hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by weight, of said parathyroid

hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23406

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/36 A61K38/00 C08L5/08 C08B37/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C08L C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	EP 0 643 963 A (MCNEIL PPC INC) 22 March 1995 (1995-03-22) page 5, line 10,11; claims 1,8-13	7,10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000	07/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Radke, M

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	SONG Y ET AL: "DRUG RELEASE AND ANTITUMOR CHARACTERISTICS OF N-SUCCINYL-CHITOSAN-MITOMYCIN C AS AN IMPLANT" JOURNAL OF CONTROLLED RELEASE,NL,ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 42, no. 1, page 93-100 XP000620286 ISSN: 0168-3659 *WHOLE DOCUMENT* -----	

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Information on patent family members

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
00537/111WO3		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23406	08/10/1999	09/10/1998
International Patent Classification (IPC) or national classification and IPC		
A61K47/36		
Applicant		
SOCIETE DE CONSEILS DE RECHERCHES ET ... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Auth rity and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28/03/2000	09.11.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Radke, M Telephone No. +49 89 2399 8677

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/23406

I. Basis f the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-10,12-26	as originally filed			
11	as received on	22/09/2000	with letter of	19/09/2000

Claims, No.:

1-9	as received on	22/09/2000	with letter of	19/09/2000

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have be n considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1, 3-7 9
	No:	Claims	2,8
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1, 3-7 9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the report

The following amendments are not directly and unambiguously disclosed in the application as originally filed. They thus contravene the requirements ot Art. 34 (2) b) PCT and are not taken into account for the purpose of this report:

(a) The insertion of "and a terminal monomer ... COH or CH_2OH" in **claims 1 and 2**.
The application as originally filed (see 2/15-3/4) only discloses that the COH or CH_2OH groups may be present in a certain position at the certain furanose-type monomer described by the formula depicted at 2/16. The insertion which allows these groups to be present at any position of any type of terminal monomer adds subject-matter to the application.

(b) The insertion of "provided that at least one of the free amines ... is acetylated with succinyl" in **claim 2**.
This insertion adds the information that exactly one of the free amines may be succinylated to the application as originally filed.

(c) The insertion of the index "2" in the third formula within the group $N-(CH_2)-CO-$ in claims 3 and 6 and on page 11.
On page 11 and in claims 6 and 9 of the application as originally four formulae are depicted of which
- the first and third one comprise a piperidine ring linked to a peptide via a $-(CH_2)-\mathbf{CO}-$ group, and
- the second and the fourth formulae comprise a piperidine ring linked to a peptide via a $-(CH_2)_2-\mathbf{SO_2}-$ group.

There is no indication or basis whatsoever in the initial version of the application that said linking goup of the third formula is to be $-(CH_2)_2-CO-$.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Cited literature

(a) Reference is made to the following documents:

D1: WO-A-96/39 160
D2: WO-A-95/04 752
D3: US-A-4 675 189
D4: EP-A-0 643 963

(b) In the following arguments, page or column A, lines B to C will be cited as A/B-C.

2. Novelty

Document **D1** discloses ionic molecular conjugates of N-acetylated derivatives of poly(2-amino-2-deoxy-D-glucose) and polypeptides.

The features of the following of the present claims are disclosed in this document as follows:

Present claims	**Disclosure in D1**
Claim 2	example 4;
claim 8	claim 14, 4/26 (and claim 9);

For this reason, the subject-matter of **claims 2 and 8** is not novel.

3. Inventive step

(a) Document **D1** is considered to represent the closest prior art.

(b) The subject-matter of **claim 1 and 9** differs from the disclosure of **D1** in that **D1** does not explicitly disclose a copolymer where the first acyl group is glutaryl and the second one is propionyl. The limited lists given at **D1**, 4/3-12, however, specifically mention glutaryl as the first acyl group and propionyl as the second one. As no special unexpected effect was demonstated by the applicant, the choice of glutaryl and propionyl as the acyl groups is considered to be obvious,

(c) Document **D1** mentions that the polypeptides may be **LH-RH**, somatostatin and biological analogs thereof (see **D1**, 4/20-34). Document **D2** gives two explicit formulae for especially preferred somatostatin analogs (see **D2**, 5/5-16 and claim 19). These formulae are identical with the third and fourth formulae in present **claims 3 and 6**. It was thus obvious for the expert to use these two preferred somatostatin analogs in **D1**. The other two formulae of present claims 3 and 6 are easily derivable from **D2** as a combination of claim 14 (which discloses the two different end groups) and 15/17 (which gives the amino acid sequence).

(d) Likewise, the **LH-RH** analogs listed in present **claims 4 and 7** are known to be LH-RH active (see **D3**, 1/59-65, 3/21-35 and especially claim 11 and example 1, where **D-Nal(2)**^{6}LH-RH is used). It was obvious for the expert to use the LH-RH peptides given in **D3** in the compositions of **D1** because both **D1** and **D3** as well as the present application deal with drug delivery systems (see 1/11-15 of the present application and **D1**, 1/5-13).

(e) The same applies to the LH-RH peptide **histrelin** (see 11/16-17 of the present application and the respective formula given in present **claims 4 and 7**). The use of this peptide in drug delivery systems is known from **D4**, claims 1 and 13. Reference is also made to **D4**, 5/10-11 where chitosan derivatives are mentioned as the other component of the drug delivery system.

(f) The subject-matter of **claim 5** is obvious in view of **D1** as the same peptide is employed (see **D1**, 12/29/31; cf. 6/14-17 of the present application).

(f) For this reason, the subject-matter of **claims 1, 3 to 7 and 9** is not based on an inventive step.

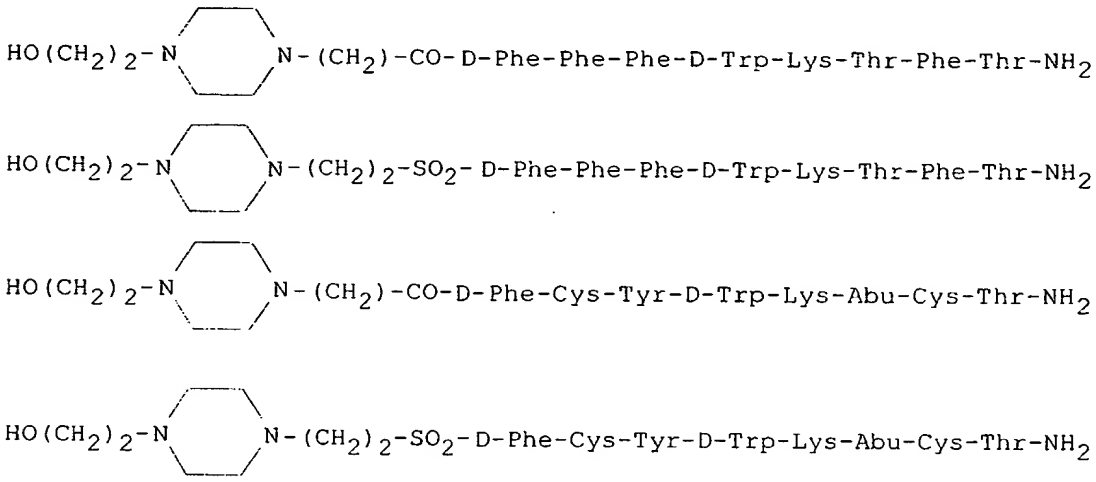
Re Item VIII

Certain observations on the international application

Clarity of the claims

Claims 3, 4 and 8 are dependent from claim 2 although they refer to peptides other than the one mentioned in claim 2. This ambiguity render claims 3, 4 and 8 unclear.

PCT Application WO 88/02756, European Application 0 329 295, and PCT Application No. WO 94/04752. An example of somatostatin agonists which contain N-terminal chemical substitutions are:



;

;

; and

or a pharmaceutically acceptable salt thereof.

Examples of specific LHRH analogues that can be incorporated in a conjugate or composition of this invention are TRYPTORELIN™ (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), buserelin ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), deslorelin ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, fertirelin ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), gosrelin ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), histrelin ([D-His(Bzl)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), leuprorelin ([D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), lutrelin ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), nafarelin ([D-Nal^6]-LHRH and pharmaceutically acceptable salts thereof.

The release of the polypeptide from the composition may be modified by changing the chemical structure of the composition. Increasing the molecular weight of the polymer will decrease the rate of peptide released from the conjugate. Increasing the number of carboxylic acid groups on the polymer will increase the amount of polypeptide ionically bound to the composition, and consequently, increase the amount of release of the peptide from the conjugate.

The release of the polypeptide may be further modulated through (a) treating the composition with soluble salts of divalent or polyvalent metallic ions of weak acids (e.g., calcium, iron, magnesium, or zinc); (b) coating the particles with a thin, absorbable layer made of a glycolide copolymer or silicone oil in a spherical, cylindrical, or planar

CLAIMS

What is claimed is:

1. A copolymer comprising an N-acylated derivative of poly(2-amino-2-deoxy-D-glucose), wherein between 1 and 50 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) are acylated with a first acyl group, said first acyl group is COE_1 where E_1 is selected from the group consisting of C_{3-33} carboxyalkyl, C_{3-33} carboxyalkenyl, C_{7-39} carboxyarylalkyl, and C_{9-39} carboxyarylalkenyl, and between 50 and 99 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) are acylated with a second acyl group, said second acyl group is COE_2 where E_2 is selected from the group consisting of C_{1-30} alkyl, C_{2-30} alkenyl, C_{6-37} arylalkyl, and C_{8-37} arylalkenyl, provided at least one of the free amines of said poly(2-amino-2-deoxy-D-glucose) is acylated with said first acyl group.

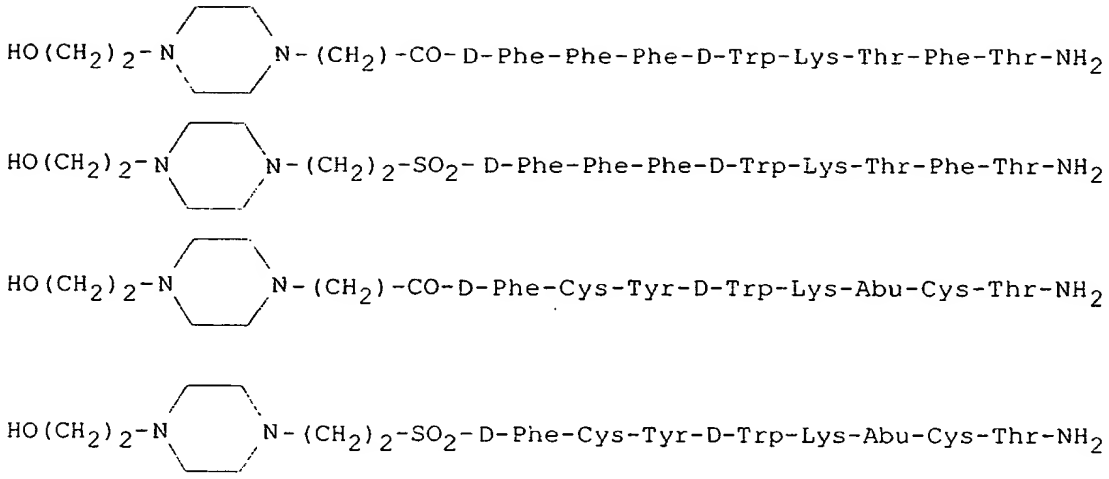
2. A copolymer of claim 1, wherein said first acyl group is COE_1 where E_1 is C_3-C_{33} carboxyalkyl.

3. A copolymer of claim 2, wherein said first acyl group is glutaryl.

4. A copolymer of claim 3, wherein said second acyl group is propionyl and R_7 is COH or CH_2OH.

5. A composition comprising said copolymer of claim 1 and H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 or a pharmaceutically acceptable salt thereof, wherein the two Cys are bonded by a disulfide bond, where at least 50 percent, by weight, of H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer, wherein said first acyl group is succinyl and said second acyl group is acetyl and R_7 is COH or CH_2OH.

6. A composition comprising said copolymer of claim 1 and a peptide selected from the group consisting of



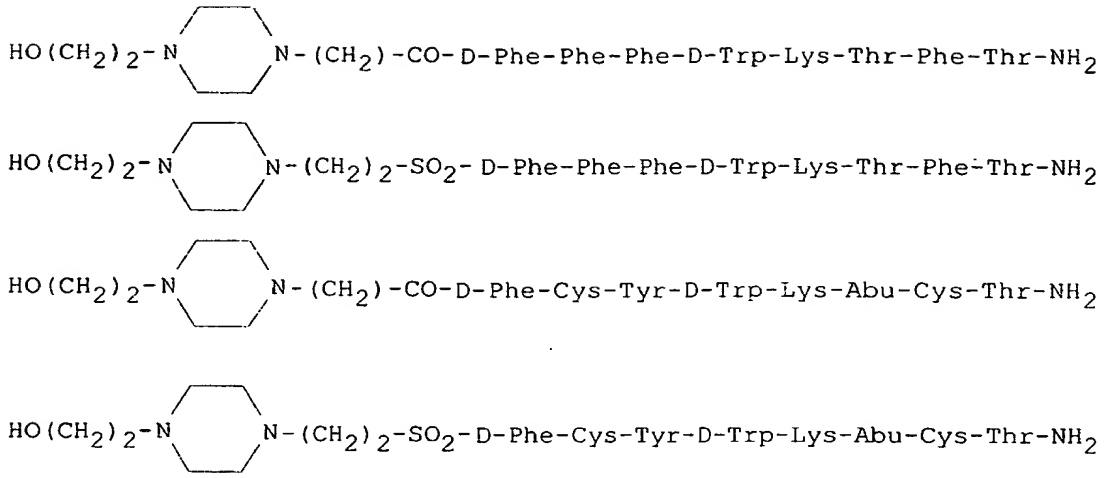
; and

or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof present in said composition is ionically bound to said copolymer, wherein said first acyl group is succinyl and said second acyl group is acetyl and R_7 is COH or CH_2OH.

7. A composition comprising said copolymer of claim 1 and a peptide selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), and ([D-Nal^6]-LHRH, or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer, wherein said first acyl group is succinyl and said second acyl group is acetyl and R_7 is COH or CH_2OH.

8. A composition comprising said copolymer of claim 4 and H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, wherein the two Cys are bonded by a disulfide bond, where at least 50 percent, by weight, of H-β-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, present in said composition is ionically bound to said copolymer.

9. A composition comprising said copolymer of claim 4 and a peptide selected from the group consisting of



; and

or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof present in said composition is ionically bound to said copolymer.

10. A composition comprising said copolymer of claim 4 and a peptide selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), and ([D-Nal^6]-LHRH, or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

11. A composition comprising said copolymer of claim 1 and parathyroid hormone, an analogue thereof or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of parathyroid hormone, an analogue thereof or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer, wherein said first acyl group is succinyl and said second acyl group is acetyl and R_7 is COH or CH_2OH.

12. A composition comprising said copolymer of claim 4 and parathyroid hormone, an analogue thereof or a pharmaceutically acceptable salt thereof, where at least 50 percent, by weight, of parathyroid hormone, an analogue or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

YR1

TENT COOPERATION TREAT

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
FISH & RICHARDSON P.C.
Attn. Tsao, Y. Rocky
225 Franklin Street
Boston, Massachusetts 02110-2804
UNITED STATES OF AMERICA

RECEIVED
FEB 14 2000
FISH & RICHARDSON, P.C.
BOSTON OFFICE

Date of mailing *(day/month/year)* 07/02/2000	
Applicant's or agent's file reference 00537/111W03	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 99/23406	International filing date *(day/month/year)* 08/10/1999
Applicant SOCIETE DE CONSEILS DE RECHERCHES ET..et al.	Search repart 4/7/00 Foreign ant 5/7/00 kad

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46).

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

Docketed By Practice Systems
SEARCH REPORT-RSPN 4/7/00
FOREIGN ANT (003) 5/7/00
Initials: KYM
Record:

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Véronique Baillou

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if th language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

TENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
00537/111W03		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 23406	08/10/1999	09/10/1998
Applicant		
SOCIETE DE CONSEILS DE RECHERCHES ET..et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to b published with the abstract is Figure No. _____

   [ ] as suggested by th applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

[redacted]tional Application No
PCT/US 99/23406

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/36 A61K38/00 C08L5/08 C08B37/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C08L C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39160 A (SHALABY SHALABY W ;JACKSON STEVEN A (US); IGNATIOUS FRANCIS (US);) 12 December 1996 (1996-12-12) page 4, line 3-26; claims 1,6,8; example 4 page 8, line 24-28	1-5,8, 11,12
Y		6,7,9,10
Y	US 4 675 189 A (KENT JOHN S ET AL) 23 June 1987 (1987-06-23) cited in the application claim 11; example 1	7,10
Y	EP 0 643 963 A (MCNEIL PPC INC) 22 March 1995 (1995-03-22) page 5, line 10,11; claims 1,8-13	7,10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000	07/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Radke, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 04752 A (BIOMEASURE INC) 16 February 1995 (1995-02-16) page 4, line 16-19; claim 19 page 15, line 17	6,9
A	SONG Y ET AL: "DRUG RELEASE AND ANTITUMOR CHARACTERISTICS OF N-SUCCINYL-CHITOSAN-MITOMYCIN C AS AN IMPLANT" JOURNAL OF CONTROLLED RELEASE,NL,ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 42, no. 1, page 93-100 XP000620286 ISSN: 0168-3659 *WHOLE DOCUMENT*	

INTERNATIONAL SEARCH REPORT

...on on patent family members

...ional Application No
PCT/US 99/23406

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639160 A	12-12-1996	US 5665702 A	09-09-1997
		AU 5878996 A	24-12-1996
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		ZA 9405966 A	26-06-1995

PATENT COOPERATION TREAT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

TSAO, Y. Rocky
FISH & RICHARDSON P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
ETATS-UNIS D'AMERIQUE

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BOSTON OFFICE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	09.11.2000
Applicant's or agent's file reference: 00537/111WO3	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23406	08/10/1999	09/10/1998

Applicant: SOCIETE DE CONSEILS DE RECHERCHES ET ... et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

* No Docketing Required *
Reviewed By Practice Systems
Initials: LXA
Reviewed By Billing Secretary
Initials:

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Aperribay, I Tel.+49 89 2399-8154

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
00537/111WO3		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23406	08/10/1999	09/10/1998
International Patent Classification (IPC) or national classification and IPC A61K47/36		
Applicant SOCIETE DE CONSEILS DE RECHERCHES ET ... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28/03/2000	09.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Radke, M Telephone No. +49 89 2399 8677

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/23406

I. Basis of th r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-10,12-26	as originally filed			
11	as received on	22/09/2000	with letter of	19/09/2000

Claims, No.:

1-9	as received on	22/09/2000	with letter of	19/09/2000

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1, 3-7 9
	No:	Claims 2,8
Inventive step (IS)	Yes:	Claims
	No:	Claims 1, 3-7 9
Industrial applicability (IA)	Yes:	Claims 1-9
	No:	Claims

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the report

The following amendments are not directly and unambiguously disclosed in the application as originally filed. They thus contravene the requirements ot Art. 34 (2) b) PCT and are not taken into account for the purpose of this report:

(a) The insertion of "and a terminal monomer ... COH or CH_2OH" in **claims 1 and 2**.
The application as originally filed (see 2/15-3/4) only discloses that the COH or CH_2OH groups may be present in a certain position at the certain furanose-type monomer described by the formula depicted at 2/16. The insertion which allows these groups to be present at any position of any type of terminal monomer adds subject-matter to the application.

(b) The insertion of "provided that at least one of the free amines ... is acetylated with succinyl" in **claim 2**.
This insertion adds the information that exactly one of the free amines may be succinylated to the application as originally filed.

(c) The insertion of the index "2" in the third formula within the group N-(CH_2)-CO- in claims 3 and 6 and on page 11.
On page 11 and in claims 6 and 9 of the application as originally four formulae are depicted of which
- the first and third one comprise a piperidine ring linked to a peptide via a -(CH_2)-**CO**- group, and
- the second and the fourth formulae comprise a piperidine ring linked to a peptide via a -$(CH_2)_2$-**SO_2**- group.

There is no indication or basis whatsoever in the initial version of the application that said linking goup of the third formula is to be -$(CH_2)_2$-CO-.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Cited literature

(a) Reference is made to the following documents:

D1: WO-A-96/39 160
D2: WO-A-95/04 752
D3: US-A-4 675 189
D4: EP-A-0 643 963

(b) In the following arguments, page or column A, lines B to C will be cited as A/B-C.

2. Novelty

Document **D1** discloses ionic molecular conjugates of N-acetylated derivatives of poly(2-amino-2-deoxy-D-glucose) and polypeptides.

The features of the following of the present claims are disclosed in this document as follows:

Present claims	**Disclosure in D1**
Claim 2	example 4;
claim 8	claim 14, 4/26 (and claim 9);

For this reason, the subject-matter of **claims 2 and 8** is not novel.

3. Inventive step

(a) Document **D1** is considered to represent the closest prior art.

(b) The subject-matter of **claim 1 and 9** differs from the disclosure of **D1** in that **D1** does not explicitly disclose a copolymer where the first acyl group is glutaryl and the second one is propionyl. The limited lists given at **D1**, 4/3-12, however, specifically mention glutaryl as the first acyl group and propionyl as the second one. As no special unexpected effect was demonstated by the applicant, the choice of glutaryl and propionyl as the acyl groups is considered to be obvious,

(c) Document **D1** mentions that the polypeptides may be **LH-RH**, somatostatin and biological analogs thereof (see **D1**, 4/20-34). Document **D2** gives two explicit formulae for especially preferred somatostatin analogs (see **D2**, 5/5-16 and claim 19). These formulae are identical with the third and fourth formulae in present **claims 3 and 6**. It was thus obvious for the expert to use these two preferred somatostatin analogs in **D1**. The other two formulae of present claims 3 and 6 are easily derivable from **D2** as a combination of claim 14 (which discloses the two different end groups) and 15/17 (which gives the amino acid sequence).

(d) Likewise, the **LH-RH** analogs listed in present **claims 4 and 7** are known to be LH-RH active (see **D3**, 1/59-65, 3/21-35 and especially claim 11 and example 1, where **D-Nal(2)**^{6}LH-RH is used). It was obvious for the expert to use the LH-RH peptides given in **D3** in the compositions of **D1** because both **D1** and **D3** as well as the present application deal with drug delivery systems (see 1/11-15 of the present application and **D1**, 1/5-13).

(e) The same applies to the LH-RH peptide **histrelin** (see 11/16-17 of the present application and the respective formula given in present **claims 4 and 7**). The use of this peptide in drug delivery systems is known from **D4**, claims 1 and 13. Reference is also made to **D4**, 5/10-11 where chitosan derivatives are mentioned as the other component of the drug delivery system.

(f) The subject-matter of **claim 5** is obvious in view of **D1** as the same peptide is employed (see **D1**, 12/29/31; cf. 6/14-17 of the present application).

(f) For this reason, the subject-matter of **claims 1, 3 to 7 and 9** is not based on an inventive step.

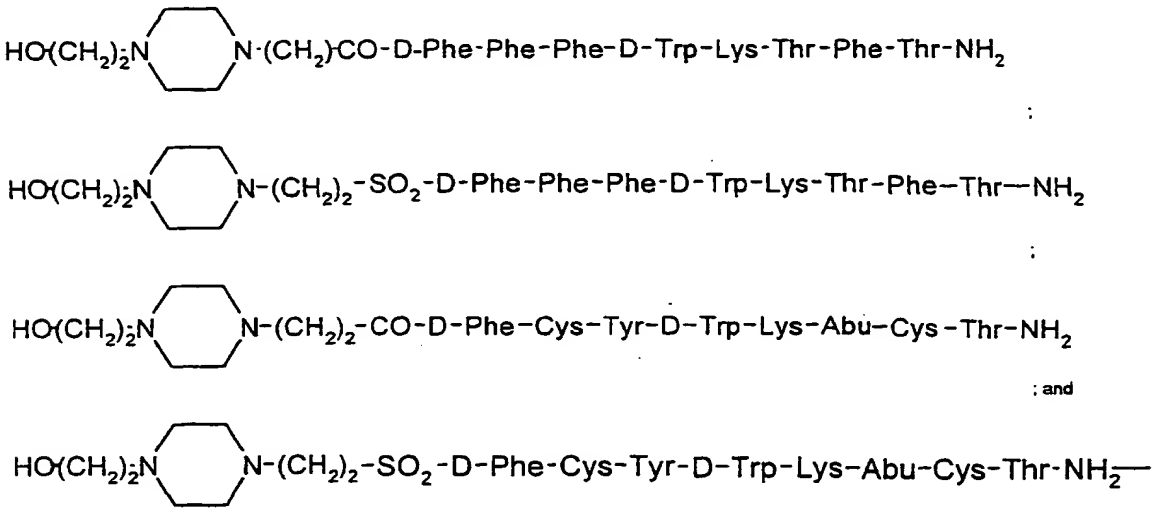
Re Item VIII

Certain observations on the international application

Clarity of the claims

Claims 3, 4 and 8 are dependent from claim 2 although they refer to peptides other than the one mentioned in claim 2. This ambiguity render claims 3, 4 and 8 unclear.

PCT Application 88/02756, European Application 0 329 295, and PCT Application No. WO 95/04752. An example of somatostatin agonists which contain N-terminal chemical substitutions are:



or a pharmaceutically acceptable salt thereof.

Examples of specific LHRH analogues that can be incorporated in a conjugate or composition of this invention are TRYPTORELIN™ (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), buserelin ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), deslorelin ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, fertirelin ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), gosrelin ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), histrelin ([D-His(Bzl)^6,des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), leuprorelin (D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), lutrelin ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), nafarelin ([D-Nal^6]-LHRH) and pharmaceutically acceptable salts thereof.

The release of the polypeptide from the composition may be modified by changing the chemical structure of the composition. Increasing the molecular weight of the polymer will decrease the rate of peptide released from the conjugate. Increasing the number of carboxylic acid groups on the polymer will increase the amount of polypeptide ionically bound to the composition, and consequently, increase the amount of release of the peptide from the conjugate.

The release of the polypeptide may be further modulated through (a) treating the composition with soluble salts of divalent or polyvalent metallic ions of weak acids (e.g., calcium, iron, magnesium, or zinc); (b) coating the particles with a thin, absorbable layer made of a glycolide copolymer or silicone oil in a spherical, cylindrical or planar

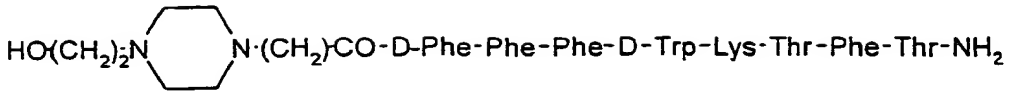
CLAIMS

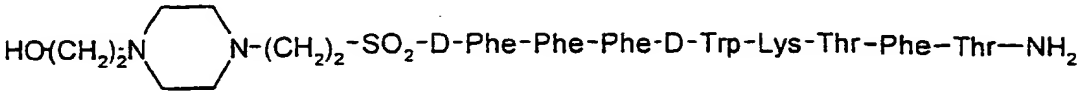
What is claimed is:

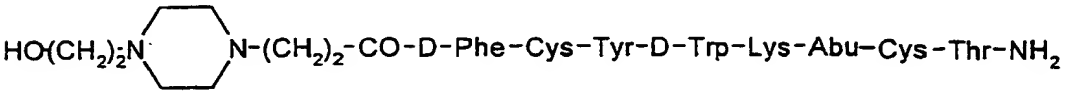
1. A copolymer comprising an N-acylated derivative of poly(2-amino-2-deoxy-D-glucose), wherein between 1 and 50 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) are acylated with glutaryl and between 50 and 99 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) are acylated with propionyl and a terminal monomer of said N-acylated derivative of poly(2-amino-2-deoxy-D-glucose) contains COH or CH_2OH.

2. A composition comprising a copolymer and a peptide, wherein said copolymer comprises an N-acylated derivative of poly(2-amino-2-deoxy-D-glucose) having between 1 and 50 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) acylated with succinyl, between 50 and 99 percent of the free amines of said poly(2-amino-2-deoxy-D-glucose) acylated with acetyl provided that at least one of the free amines of said poly(2-amino-2-deoxy-D-glucose) is acylated with succinyl, and a terminal monomer of said N-acylated derivative of poly(2-amino-2-deoxy-D-glucose) containing COH or CH_2OH, and wherein said peptide comprises H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 or a pharmaceutically acceptable salt thereof, having the two Cys of said peptide bonded by a disulfide bond and at least 50 percent by weight, of the H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 peptide, or a pharmaceutically acceptable salt thereof, present in said composition ionically bound to said copolymer.

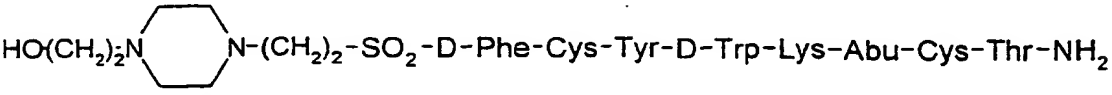
3. A composition comprising said copolymer of claim 2 and a peptide wherein said peptide is selected from the group consisting of







; and

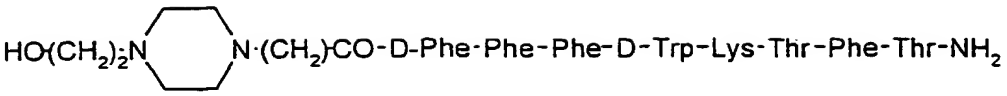


or a pharmaceutically acceptable salt thereof, having at least 50 percent, by weight, of said peptide, or a pharmaceutically acceptable salt thereof, present in said composition, ionically bound to said copolymer.

4. A composition comprising said copolymer of claim 2 and a peptide wherein said peptide is selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6,des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), (D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Nal^6]-LHRH) or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by weight, of said peptide, or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

5. A composition comprising said copolymer of claim 1 and H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, wherein the two Cys are bonded by a disulfide bond and at least 50 percent, by weight, of H-ß-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, present in said composition is ionically bound to said copolymer.

6. A composition comprising said copolymer of claim 1 and a peptide selected from th group consisting of



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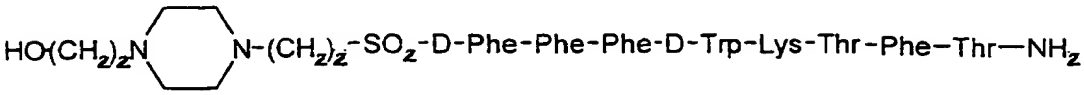
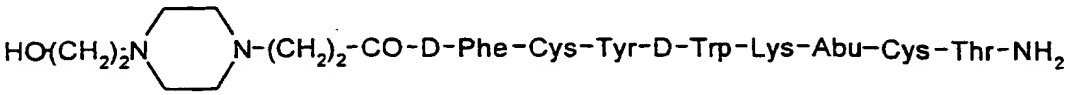
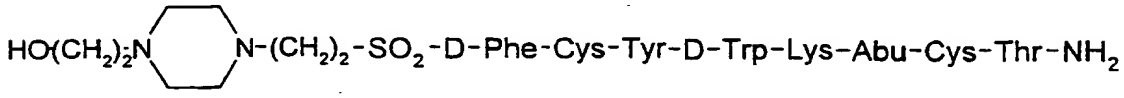




; and



or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof present in said composition is ionically bound to said copolymer.

7. A composition comprising said copolymer of claim 1 and a peptide selected from the group consisting of (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), ([D-His(Bzl)^6,des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), (D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), ([D-Nal^6]-LHRH) or a pharmaceutically acceptable salts thereof, wherein at least 50 percent, by weight, of said peptide or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

8. A composition comprising said copolymer of claim 2 and parathyroid hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by weight, of said parathyroid hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

9. A composition comprising said copolymer of claim 1 and parathyroid hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, wherein at least 50 percent, by w ight, of said parathyroid

hormone, an analogue thereof, or a pharmaceutically acceptable salt thereof, present in said composition is ionically bound to said copolymer.

M·H

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
00537/111W03		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 23406	08/10/1999	09/10/1998
Applicant		
SOCIETE DE CONSEILS DE RECHERCHES ET..et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]US 99/23406

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/36 A61K38/00 C08L5/08 C08B37/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C08L C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39160 A (SHALABY SHALABY W ;JACKSON STEVEN A (US); IGNATIOUS FRANCIS (US);) 12 December 1996 (1996-12-12) page 4, line 3-26; claims 1,6,8; example 4 page 8, line 24-28	1-5,8, 11,12
Y	---	6,7,9,10
Y	US 4 675 189 A (KENT JOHN S ET AL) 23 June 1987 (1987-06-23) cited in the application claim 11; example 1	7,10

Y	EP 0 643 963 A (MCNEIL PPC INC) 22 March 1995 (1995-03-22) page 5, line 10,11; claims 1,8-13	7,10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000	07/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Radke, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 04752 A (BIOMEASURE INC) 16 February 1995 (1995-02-16) page 4, line 16-19; claim 19 page 15, line 17	6,9
A	SONG Y ET AL: "DRUG RELEASE AND ANTITUMOR CHARACTERISTICS OF N-SUCCINYL-CHITOSAN-MITOMYCIN C AS AN IMPLANT" JOURNAL OF CONTROLLED RELEASE,NL,ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 42, no. 1, page 93-100 XP000620286 ISSN: 0168-3659 *WHOLE DOCUMENT*	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/23406

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